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Development of a Comparative Potency Method for Cancer Risk Assessment of Complex Mixtures Using Short-Term 'In vivo' and In vitro' Bioassays

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DEVELOPMENT OF A COMPARATIVE POTENCY METHOD FOR CANCER RISK ASSESSMENT OF COMPLEX MIXTURES USING SHORT-TERM *IN VIVO* AND *IN VITRO* BIOASSAYS

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A comparative potency method for cancer risk assessment has been developed based on a constant relative potency hypothesis. This method was developed and tested using data from a battery of short-term mutagenesis bioassays, animal tumorigenicity data and human lung cancer risk estimations. This data base was developed for a series of complex mixtures including emissions from coke ovens, roofing tar pots, cigarette smoke and automotive engines. The series of automobiles used in this study included both diesel- and gasoline-powered vehicles. The assumptions inherent in this method are discussed, together with the methods and data base used to test these assumptions.

INTRODUCTION

Short-term genetic bioassays are now widely used to screen both individual chemicals (e.g., pesticides and toxic substances) and complex mixtures (e.g., industrial effluents, automotive emissions) for mutagenic and potential carcinogenic activity. These bioassays are often used in a tiered or phased approach to rank substances by priority for more expensive chronic animal testing (Bridges, 1973; Waters, 1978). The results of both microbial and mammalian cell genetic bioassays are now considered by government regulatory agencies and international organizations to provide important suggestive evidence of a substance's carcinogenicity in humans (NAS, 1983; IARC, 1983).

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2. Key words: alternative energy sources, carcinogenicity, combustion emissions, comparative potency, constant relative potency, genetic bioassays.

3. Abbreviations: a, alternative; c, conventional; CHO, Chinese hamster ovary; CV, coefficient of variation; DCM, dichloromethane; DMSO, dimethylsulfoxide; IARC, International Agency for Research on Cancer; IRLG, Interagency Regulatory Liaison Group; MA, metabolic activation; NAS, National Academy of Sciences; rev, revertants; SCE, sister chromatid exchange.

Most, if not all, conventional energy combustion sources and fuels currently in use emit carcinogenic polycyclic organic compounds that are mutagenic in short-term genetic bioassays using microbial and mammalian cells (Claxton and Huisingh, 1980; Lewtas, 1982; Holmberg and Ahlborg, 1983) and that are tumorigenic in animals (Nesnow et al., 1982a,b). The primary problem associated with introducing new energy sources is whether they will alter the mutagenicity, carcinogenicity and potential human cancer risk from the resulting combustion emissions. This problem is serious, since it is not possible to collect human cancer risk data for exposures to emissions that have not been previously released to the environment. Additionally, the use of chronic animal testing for each new combustion technology developed would be prohibitively expensive and time consuming. This problem has motivated us to develop and test new risk assessment methodologies that would use short-term mutagenesis and tumorigenesis data.

This research began with a study to assess the relative human cancer risk associated with introducing diesel-powered automobiles as an alternative fuel-efficient energy source for transportation. A comparative data base (Lewtas et al., 1981; Nesnow and Lewtas, 1981) and the comparative risk assessment methodology (Albert et al., 1983) were developed. Although initially developed to compare alternative combustion sources (e.g., engines) that are powered by different fuels (e.g., diesel vs. gasoline), this risk assessment model and methodology are applicable to the evaluation of various complex mixtures. This paper describes a comparative approach to evaluating such complex mixtures using short-term genetic bioassays. The methodology and assumptions inherent in the method are first presented and discussed. The experimental methods used in sampling and bioassay of the complex mixtures are summarized. Finally, the application of this method to the comparative risk assessment of complex mixtures is presented.

COMPARATIVE POTENCY METHOD

The comparative potency method for cancer risk assessment is based on the hypothesis that there is a constant relative potency between two different carcinogens across different bioassay systems. The mathematical expression for the constant relative potency model is:

$$\text{Relative Potency in Bioassay}_{(1)} = (k)\ \text{Relative Potency in Bioassay}_{(2)}$$

This assumption is implicit in any comparison that uses the relative toxicity of two substances in animals to determine which substance would most likely be less toxic to man. This constant relative potency assumption is a hypothesis that can be tested, if the relative potency of two carcinogens in humans can be determined and compared to the relative potency in a series of *in vitro* and *in vivo* bioassays. The test of this model is whether there is a constant relationship (k) between the relative potencies in the two bioassays being compared such that:

$$\frac{\text{Relative Potency in Bioassay}_{(1)}}{\text{Relative Potency in Bioassay}_{(2)}} = \text{constant (k)}.$$

We have tested this hypothesis for three complex organic emissions from a coke oven, roofing tar pot and cigarettes using human lung cancer data from epidemiologic studies of humans exposed to these emissions (Albert et al., 1983). The relative potencies for coke oven emissions compared to roofing tar emissions were similar and ranged from 0.2-1.4 across data from humans, mouse skin tumor-initiation assays and selected short-term genetic bioassays as shown in Table 1. When the relative potency of cigarette smoke was compared to that of roofing tar or coke ovens, however, the short-term bioassays showed a much higher relative potency than the human and mouse skin tumor-initiation bioassay. A series of diesel and gasoline automotive emissions, for which no human data were available, was also tested in the battery of *in vivo* and *in vitro* bioassays. The relative potency of the automotive samples across these bioassays was, however, relatively constant as shown in Table 2. High correlations were observed between the mutagenic activity of these emissions and their tumor-initiating activity (Lewtas, 1982) as shown in Table 2. The best correlations were observed in the mouse lymphoma bioassay ($r^2 = 0.95$) and the Ames, TA98, -MA bioassay ($r^2 = 0.90$).

This method was initially applied to estimating a human lifetime cancer risk (unit risk) associated with emissions from a series of diesel automobiles by comparison to the human cancer unit risk from coke oven, roofing tar and cigarette emissions according to the following equation (e.g., for coke oven emissions):

$$\text{relative human risk } \left(\frac{\text{diesel}}{\text{coke oven}}\right) = (k) \text{ relative bioassay potency } \left(\frac{\text{diesel}}{\text{coke oven}}\right).$$

The relative potency in the mouse skin tumor-initiation bioassay was used to estimate the cancer risk from diesel emissions. This approach to applying the comparative potency method assumes that when comparing between one of the three human carcinogens and the automotive emissions, the mouse skin best predicts the relative potency in humans. This assumption is supported by the correlation ($r^2 = 0.96$) between the relative potencies in mouse skin tumor-initiating activity and the human lung cancer unit risks. Within the automotive emissions studies, however, it was assumed that an average of the relative potencies across the three short-term bioassays could be used to estimate the human risk, since the relative potencies in the short-term bioassays correlated well with the mouse skin tumor-initiating activity (Albert et al., 1983) as shown in Table 2.

Since the relative mutagenic potency of these automotive emissions correlates so well with the relative tumorigenic activity, it is possible to calculate a proportionality constant to use in estimating the human health risk from any one of these emissions using mutagenicity data as shown in Table 3. This calculation of k is also a test of the constant relative potency hypothesis, with the relative potency constant ranging from 0.023 to 0.11, or approximately fivefold.

TABLE 1
Comparison of Relative Potencies of Emission Extracts in Humans and Bioassay Systems[a]

Emission Extracts	Human Lung Cancer	Mouse Skin Tumor Initiation	Mutation in Mouse Lymphoma Cells (+MA)	Mutation in Ames TA98 (+MA)
Coke Oven Topside	1.0	1.0	1.0	1.0
Roofing Tar	0.39	0.20	1.4	0.78
Cigarette	0.0024	0.0011	0.066	0.52
Diesel (Nissan)		0.28	0.24	12.

[a]The relative bioassay potencies were determined for each of the bioassay systems by dividing the absolute activity (e.g., lung cancer risk, tumor initiation activity or mutagenicity) of each emission by the activity of the coke oven emission within that bioassay (Albert et al., 1983). The mouse skin tumor initiation data (Nesnow et al., 1982a,b), mouse lymphoma data (Mitchell et al., 1981), and Ames data (Claxton, 1981) are reported and evaluated elsewhere (Lewtas, 1982; Albert et al., 1983). The collection and preparation of emission extracts have been previously reported (Lewtas et al., 1981).

TABLE 2
Comparison of Relative Potencies of Diesel and Gasoline Emission Extracts in Several Bioassay Systems[a]

Emission Extracts	Mouse Skin Tumor Initiation	Mutation in Mouse Lymphoma Cells		SCE in CHO Cells		Mutation in Ames TA98	
		(-MA)	(+MA)	(-MA)	(+MA)	(-MA)	(+MA)
DIESELS							
Nissan	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Volkswagen Rabbit	0.41	0.23	0.25	0.25	0.42	0.34	0.23
Oldsmobile	0.53	0.29	0.45	Neg	0.24	0.19	0.11
Caterpillar	Neg	0.06	0.022	0.037	Neg	0.034	0.023
GASOLINE CATALYST							
Mustang II	0.29	0.90	0.38	0.25	NT	0.26	0.26
Correlation (r^2) with mouse skin tumor initiation	-	0.95	0.95	0.83	0.83	0.90	0.72

[a]The relative potencies were determined by dividing the tumorigenicity or mutagenicity (linear slope of the dose-response curve) of each emission by the activity of the Nissan diesel within that bioassay. Several emissions were either negative (Neg) or not tested (NT) due to insufficient amounts of sample. The data for each assay are reported elsewhere (Lewtas, 1982; Nesnow et al., 1982a,b; Albert et al., 1983; Claxton, 1981; Mitchell et al., 1981). The generation, collection and preparation of these automotive emissions have been previously reported (Lewtas, 1982; Lewtas et al., 1981).

The major assumption and main source of uncertainty in this comparative potency method is the validity of the constant relative potency hypothesis. Interspecies differences in absorption and metabolism, which present the most difficult problems in extrapolating from animals to humans, may also affect the relative carcinogenic potency. By developing and evaluating a comparative data base across human, animal and short-term bioassays for the same substances, we can test these assumptions. The constant relative potency model does not assume *a priori* that the carcinogens being compared are chemically similar. The coke oven, roofing tar, cigarette smoke and automotive emission samples are similar in that they all contain polycyclic organic compounds that cause frameshift mutations in *Salmonella typhimurium* (Claxton and Huisingh, 1980). However, these four emissions differ in their MA requirements (Claxton, 1981), and upon chemical fractionation and characterization, significant differences are observed in the specific mutagenic/carcinogenic chemicals present (Lewtas, 1984). The similarity in chemical composition of the different diesel automobile emissions may, however, be the basis for the constant relative potency observed across the short-term bioassays and animal tumorigenicity studies for this series of emissions. The mutagens and carcinogens present in these mixtures may differ only in their concentration.

EXPERIMENTAL METHODS

Emission Sample Collection and Extraction Methods. Combustion emissions containing both gaseous and particulate matter are typically collected by filtration or electrostatic precipitation after dilution and cooling (Bradow, 1982; Schuetzle, 1983) or are directly collected by condensation (Grimmer et al., 1973). The automotive emissions described here, as well as the residential oil and wood combustion emissions, were collected using a modification of the standard dilution tunnel method (Lewtas et al., 1981; Bradow, 1982) in which the exhaust is collected on large 20-in by 20-in Teflon-coated Pallflex T60-A20 filters. The collection methods for the coke oven, roofing tar and cigarette smoke condensate have been described elsewhere (Lewtas et al., 1981). The particulate samples were Soxhlet-extracted with DCM for 48 hr and filtered (0.2 m pore), and the DCM was removed by evaporation and solvent-exchanged into DMSO for all the bioassays discussed here except for the mouse skin tumorigenesis assay, in which acetone was used as the solvent.

Bioassay Methods. The short-term genetic bioassays in which dose-dependent and reproducible increases in response were obtained for many of the combustion emission samples evaluated included:

1. Reverse mutation in *Salmonella typhimurium* TA98 (Ames bioassay) (Claxton, 1981).
2. Forward mutation at the thymidine kinase locus in L5178Y mouse lymphoma cells (mouse lymphoma bioassay) (Mitchell et al., 1981).
3. SCE in CHO cells (SCE bioassay) (Mitchell et al., 1981).

For these bioassays, one or more preliminary range-finding tests were performed to determine the optimum dose range for defining the slope of the dose-response curve. The bioassays were generally performed with duplicate or triplicate treatments at five to seven doses. The bioassays were all performed in the presence and absence of an Aroclor-induced rat liver S9 MA system. The linear slope of the dose-response curve determined from simple regression analysis was used for the comparative potency analysis as previously described (Albert et al., 1983). The fit to linear response was generally good, and the r^2 for each linear regression analysis is reported elsewhere (Lewtas, 1982).

Mouse skin tumorigenesis assays that have had considerable use in determining the tumorigenic and carcinogenic effects of chemicals and complex mixtures using SENCAR mice were performed as previously described (Nesnow et al., 1982a). The assay used five single doses (80 mice/dose), and animals were scored individually at 6 mo for papillomas and at 1 yr for squamous cell carcinomas (Nesnow et al., 1982b). The carcinoma responses in the initiation assay were correlated with the papilloma responses as described by Nesnow et al. (1982b); only the papilloma responses were used in the comparative potency analysis (Albert et al., 1983).

COMPARATIVE ASSESSMENT OF COMPLEX MIXTURES FROM ALTERNATIVE ENERGY SOURCES

Short-term bioassays have proved useful in evaluating the effects of various engines, fuels and control technologies on the mutagenicity of emissions. To draw meaningful conclusions from such comparisons, however, the normal day-to-day variations in the emissions were studied for one engine under standard conditions (Claxton and Kohan, 1981). The CV for the particulate emission rates, percentage of organic extractables and mutagenic activity ranged from 0.07 to 0.11. To compare the mutagenicity of particulate emissions from different sources or fuels, the percentage of organic materials extractable from the particles and the vehicle's particulate emission rate must also be included in the analysis. The final rate of mutagenic emissions is determined from all three parameters.

Although pure compounds have been reported to range in carcinogenic and mutagenic potency over six orders of magnitude (Meselson and Russell, 1977), the relative potency of combustion emissions ranged from only a fivefold difference in the mouse skin tumorigenesis assay to a 150-fold difference in the mouse lymphoma gene mutation assay. The Ames *Salmonella typhimurium* mutagenesis assay showed approximately two orders of magnitude difference (100- to 110-fold) in mutagenic activity from the weakest to the most potent emissions sample when measured per mass of extractable organics (e.g., rev/μg). The emissions rates (rev/km or rev/J) for the particle-bound organics, however, differed in some cases by as much as five orders of magnitude (10^5). To compare the mutagenicity of emissions from various sources directly, the rates of mutagenic emissions must be compared on the same basis (e.g.,

TABLE 3
Relative Potencies of Automotive Emission Compared to Coke Over Emission

	Relative Potency		
Emission Sample	Mouse Skin Tumorigenesis [a]	Ames [b]	k [c]
Coke Oven Topside	1.0	1.0	-
Diesel Nissan	0.28	12.0	0.023
Diesel Volkswagen Rabbit	0.11	2.7	0.041
Diesel Oldsmobile	0.15	1.4	0.11
Gasoline Mustang II	0.08	3.2	0.025
		Ave =	0.05

[a]Relative potency determined by dividing the tumorigenic potency of each emission by the tumorigenic potency of the coke over topside sample.

[b]Relative potency determined by dividing the mutagenic potency in the Ames *Salmonella typhimurium* bioassay in the presence of metabolic activation of each emission by the mutagenic potency of the coke topside sample.

[c] $k = \frac{\text{relative potency in mouse skin}}{\text{relative potency in Ames}}$

fuel consumption, distance driven, energy consumption or yield). To compare rates of mutagenic emissions from mobile sources, we have compared the mutagenic activity per km, as shown in Table 4. The rates of mutagenic emissions from stationary sources are compared on the basis of fuel or energy consumption, as shown in Table 5.

This comparative potency method provides a framework for evaluating alternative energy sources, fuels or control techniques by comparing them to the conventional technologies in short-term genetic bioassays. It is possible to extend the method described here for estimating lung cancer unit risks for diesel and gasoline automotive emissions to unit risk estimates for various combustion emissions or other complex mixtures through the data base established linking human, animal and short-term genetic bioassay data. A simplified comparative approach to evaluating alternative energy sources is to use parallel bioassay studies of the alternative (a) and conventional (c) source emissions and to determine a relative risk by direct comparison as follows:

$$\text{Increased Risk (a/c)} = \text{Relative Bioassay Potency (a/c)}.$$

Since there is no *one* conventional, standard, petroleum-derived fuel nor *one* standard combustion source, such studies need to consider the range of mutagenic and carcinogenic potency between different conventional sources and fuels. The establishment of such a range could then serve as a guide for evaluating alternative fuels or sources. For example, if the relative potency of the alternative source or fuel emissions fell within the range for the conventional fuels, one could assume no increased human risk. The battery of bioassays to be used in such a study would depend on the availability of

TABLE 4
Comparative Mutagenic Rates for Transportation Combustion Sources[a]

Vehicles	Fuel type	Mutagenicity of Organics: Ames TA98 (+MA)	Mutagenicity of Organics: Mouse Lymphoma (+MA)	Organic Emission Rate (mg/km)	Mutagenic Emission Rates (activity/km): Ames TA98 ($\times 10^5$)	Mutagenic Emission Rates (activity/km): Mouse Lymphoma ($\times 10^5$)
Diesel engine						
Car (Mercedes)	diesel	12.0	1.5	19.2	2.4	0.30
Truck (Ford/Cat)	diesel	1.7	0.28	302.0	5.3	0.85
Bus (GM)	diesel	0.1	0.35	357.0	.37	1.3
Gasoline engine						
Noncatalyst (Ford Van)	unleaded gasoline	32.0	5.7	5.7	1.8	0.32
Catalyst (Mustang II)	leaded gasoline	3.5	1.1	1.4	.05	0.02

[a]The mutagenicity of the organics in the Ames bioassay is expressed in rev/μg and in the mouse lymphoma bioassay as mutants/10^6 survivor/μg/-ml (mut. freq./μg/ml). The mutagenic emission rates were determined as described in Table 3. The methods for collecting these emissions and performing the mutagenesis bioassays have been reported elsewhere (Lewtas, 1982; Lewtas et al., 1981; Lewtas, 1984).

TABLE 5
Comparative Mutagenic Emission Rates for Stationary Energy Combustion Sources[a]

Residential	Fuel Type	Mutagenicity of Organics Ames TA98 (rev/μg)	Organic Emission Rate (ng/J)	Mutagenic Emission Rate (rev/J) ($\times 10^3$)
Woodstove (airtight)	Pine	1.3	508.0	660.0
Woodstove (airtight)	Oak	0.9	187.0	168.0
Oil furnace #1	Oil #2	2.0	0.5	1.0
Oil furnace #2	Oil #2	5.1	1.5	7.6
Utility power plants				
Conventional	coal	3.1	0.01	0.031
Fluidized-bed	coal	9.4	0.03	0.28

[a]The mutagenic emission rates were determined as described in Table 3. The mutagenicity of these stationary source combustion emissions have been reviewed elsewhere (Lewtas, 1984). The mutagenicity of the woodstove and oil furnace emissions is shown in the presence of MA, and the utility coal power plant emissions, in the absence of MA.

emission samples, resources and time. As the results from more bioassays are used, however, confidence in the relative potency estimates should increase.

The range of mutagenic activity (e.g., rev/μg) and mutagenic emission rate (e.g., rev/km) in a battery of short-term genetic bioassays have been compared among a series of petroleum-derived fuels that meet the specifications for no. 2 diesel fuel for automobiles. Within these specifications, however, is a range of fuels, from minimum quality fuels (high nitrogen, sulfur and aromatic content and low cetane value) to premium quality fuels (low sulfur, nitrogen, and aromatic content and high cetane value). Table 6 shows the range in mutagenic emission rates for three short-term bioassays when three no. 2 diesel fuels were combusted in two vehicles. The mutagenic emission rates generally ranged from twofold to fivefold except for the SCE assay for the truck emissions in the absence of S9, which showed a 31-fold range. The combustion emissions from synfuels with chemical compositions significantly different from petroleum-derived fuels could be evaluated in the same bioassays to determine if the relative risk from these emissions is increased by a factor significantly greater than the differences observed among currently used petroleum-derived fuels.

This direct comparative analysis of mutagenic emissions rates provides a simple means for evaluating alternative energy sources. It is apparent from this data base that the mutagenic emission rate (e.g., rev/kg fuel) may vary over a wider range than the mutagenicity or carcinogenicity of the emissions (e.g., rev/mg particles). Therefore,

TABLE 6
Range of Mutagenic Emission Rates from No. 2 Diesel Petroleum Fuels[a]

		Mutagenic Activity/km					
Diesel Vehicle	MA	Mutation in Ames TA98 ($\times 10^5$)	Range	Mutation in Mouse Lymphoma Cells ($\times 10^5$)	Range	SCE in CHO Cells ($\times 10^4$)	Range
Truck (Ford/Cat)	-	6.8-14.	(2.1x)	0.91-3.5	(3.8x)	0.21-6.6	(31x)
	+	5.2-17.	(3.3x)	0.85-1.4	(1.6x)	1.2 -2.1	(1.7x)
Bus (GM/Detroit)	-	0.22-1.1	(5.0x)	----		0.91-2.8	(3.1x)
	+	0.37- .86	(2.3x)	1.3-4.3	(3.3x)	0.72-1.7	(2.4x)

[a]The mutagenic emission rate, mutagenic activity/km, was determined from the mutagenic activity dose-response slope of the particulate emission extracts, the percent organic extractables and particulate emission rates for each vehicle. The mutagenic emission rate units for each bioassay are as follows: Ames (revertants/km x 10^5), mouse lymphoma (mutants/10^6 survivors/μg/ml/km x 10^5), SCE in CHO cells (SCE/cell/μg/ml/km x 10^4). The range in activity between the different fuels is shown in parentheses, e.g. (2.1x) for each bioassay. The truck (Ford/Cat) was a heavy-duty Bob Tail Van (LN-7000) with a Caterpillar 3208 engine. The bus (GM/Detroit) was a General Motors city bus with a Detroit diesel DD8V-71 engine. Both vehicles were operated on the 83 transient cycle with premium (EM-242F), average (EM-239F) and minimum quality (EM-241F) petroleum-derived diesel fuels. The specifications and chemical composition of these fuels have been previously reported (Huisingh et al., 1979).

when directly comparing sources, the mutagenic or tumorigenic emission rate should be compared.

The increased or decreased human cancer risk from combustion emissions may be of greater concern than the absolute risk from these emissions when the effects of alternative energy technologies and fuels are considered. The most valuable data for determining this increased or decreased risk are the comparative bioassay data on emissions from the complex mixture being evaluated or the comparative bioassay data on the conventional and alternative technology being evaluated. Such studies should be useful in providing direction for engineers and chemists to design alternative energy sources and fuels that produce less mutagenic and potentially less carcinogenic emissions.

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REFERENCES

ALBERT, R.E., LEWTAS, J., NESNOW, S., THORSLUND, T.W., and ANDERSON, E. (1983). Comparative potency method for cancer risk assessment: Application to diesel particulate emissions. Risk Anal. 3:101-117.

BRADOW, R.L. (1982). Diesel particle and organic emissions: Engine simulation, sampling and artifacts. In: *Toxicological Effects of Emissions from Diesel Engines* (J. Lewtas, Ed.) pp. 33-47, Elsevier Science Publishing Co., Inc., New York.

BRIDGES, B.A. (1973). Some general principles of mutagenicity screening and a possible framework for testing procedures. Environ. Health Persp. 6:221-227.

CLAXTON, L.D., and HUISINGH, J.L. (1980). Comparative mutagenic activity of organics from combustion sources. In: *Pulmonary Toxicology of Respirable Particles* (C.L. Sanders, F.T. Cross, G.E. Dagle and J.A. Mahaffey, Eds.) pp. 453-465, Department of Energy, U.S. Government Printing Office, CONF-791002.

CLAXTON, L.D. (1981). Mutagenic and carcinogenic potency of diesel and related environmental emissions: *Salmonella* bioassay. Environ. Int. 5:389-391.

CLAXTON, L., and KOHAN, M. (1981). Bacterial mutagenesis and the evaluation of mobile-source emissions. In: *Short-term Bioassays in the Analysis of Complex Environmental Mixtures II* (M.D. Waters, S.S. Sandhu, J. Huisingh, L. Claxton and S. Nesnow, Eds.) pp. 299-317, Plenum Press, New York.

GRIMMER, G., HILDEBRANDT, A., and BOHNKE, H. (1973). Investigations on the carcinogenic burden by air pollution in man. II. Sampling and analytics of polycyclic aromatic hydrocarbons in automobile exhaust gas. I. Optimization of the collecting arrangement. Zbl. Bakt. Hyg., I. Abt. Orig. B. 158:22-34.

HOLMBERG, G., and AHLBORG, U. (1983). Consensus report: Mutagenicity and carcinogenicity of car exhausts and coal combustion emissions. Environ. Health Persp. 47:1-30.

HUISINGH, J., BRADOW, R., JUNGERS, R., CLAXTON, L., ZWEIGINGER, R., TEJADA, S., BUMGARNER, J., DUFFIELD, F., WATERS, M., SIMMON, V.F., HARE, C., RODRIGUEZ, C., and SNOW, L. (1979). Application of bioassays to the characterization of diesel particle emissions. In: *Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures* (M.D. Waters, S. Nesnow, J.L. Huisingh, S.S. Sandhu and L. Claxton, Eds.) pp. 381-418, Plenum Press, New York.

IARC. (1983). *IARC Internal Technical Report No. 83/001.* IARC, Lyon, France.

LEWTAS, J., BRADOW, R.L., JUNGERS, R.H., HARRIS, B.D., ZWEIDINGER, R.B., CUSHING, K.M., GILL, B.E., and ALBERT, R.E. (1981). Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: Study design, sample generation, collection, and preparation. Environ. Int. **5**:383-387.

LEWTAS, J. (1982). Mutagenic activity of diesel emissions. In: *Toxicological Effects of Emissions from Diesel Engines* (J. Lewtas, Ed.) pp. 243-264, Elsevier Science Publishing Co., Inc., New York.

LEWTAS, J. (1984). Combustion emissions: Characterization and comparison of their mutagenic and carcinogenic activity. In: *Carcinogens and Mutagens in the Environment*, Vol. IV (H.F. Stich, Ed.), CRC Press, Boca Raton, Florida.

MESELSON, M., and RUSSELL, K. (1977). Comparisons of carcinogenic and mutagenic potency. In: *Origins of Human Cancer*, Book C (H.H. Hiatt, J.D. Watson and J.A. Winsten, Eds.) pp. 1473-1481, Cold Spring Harbor Lab., New York.

MITCHELL, A.D., EVANS, E.L., JOTZ, M.M., RICCIO, E.S., MORTELMANS, K.E., and SIMMON, V.F. (1981). Mutagenic and carcinogenic potency of *in vitro* mutagenesis and DNA damage. Environ. Int. **5**:393-402.

NAS. (1983). Risk Assessment in the Federal Government: *Managing the Process.* Committee on the Institutional Means for Assessment of Risk to Public Health, NAS-NRC, 189 pp.

NESNOW, S., and LEWTAS, J. (1981). Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: Summary and discussion of the results. Environ. Int. **5**:425-429.

NESNOW, S., TRIPLETT, L., SLAGA, T.J. (1982a). Comparative tumor-initiating activity of complex mixtures on mouse skin. J. Natl. Cancer Inst. **68**:829-834.

NESNOW, S., EVANS, C., STEAD, A., CREASON, J., SLAGA, T.J., and TRIPLETT, L.L. (1982b). Skin Carcinogenesis studies of emissions extracts. In: *Toxicological Effects of Emissions from Diesel Engines* (J. Lewtas, Ed.), pp. 295-320, Elsevier Science Publishing Co., Inc., New York.

SCHUETZLE, D. (1983). Sampling of vehicle emissions for chemical analysis and biological testing. Environ. Health Persp. **47**:65-80.

WATERS, M.D. (1978). A phased approach to the bioscreening of emissions and effluents from energy technologies. In: *Proceedings of Symposium on Potential Health and Environmental Effects of Synthetic Fossil Fuel Technologies*, pp. 143-152. September 25-28, 1978, Gatlinburg, Tennessee, First Annual Oak Ridge National Laboratory Life Sciences Symposium, Department of Energy (CONF-780903).